

The University of Chicago

THE DUAL ANTIBODY BASIS OF
ACQUIRED IMMUNITY IN
TRICHINOSIS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1941

By

JOSÉ OLIVER-GONZÁLEZ

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
JOURNAL OF INFECTIOUS DISEASES
Vol. 69, November-December, 1941

The University of Chicago

THE DUAL ANTIBODY BASIS OF
ACQUIRED IMMUNITY IN
TRICHINOSIS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1941

By
JOSÉ OLIVER-GONZÁLEZ

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
JOURNAL OF INFECTIOUS DISEASES
Vol. 69, November-December, 1941



THE DUAL ANTIBODY BASIS OF ACQUIRED
IMMUNITY IN TRICHINOSIS

JOSÉ OLIVER-GONZÁLEZ

FROM THE DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY, UNIVERSITY OF CHICAGO, AND THE
SCHOOL OF TROPICAL MEDICINE, UNIVERSITY OF PUERTO RICO, UNDER THE AUSPICES OF
COLUMBIA UNIVERSITY

Reprinted from the Journal of Infectious Diseases
November-December, 1941, Vol. 69, pp. 254-270

GEORGE BANTA PUBLISHING CO., MENASHA, WIS.

THE DUAL ANTIBODY BASIS OF ACQUIRED IMMUNITY IN TRICHINOSIS

JOSÉ OLIVER-GONZÁLEZ

From the Department of Bacteriology and Parasitology, University of Chicago, and the School of Tropical Medicine, University of Puerto Rico, under the auspices of Columbia University

The following experiments were designed to demonstrate more specific differences between the antibodies developed by the host against the adults and larvae of *Trichinella spiralis* as well as to determine the relative importance of these antibodies in acquired immunity. The prevailing idea is that immunity to *T. spiralis* is directed towards preventing the establishment of adult worms in the intestine. The fact that this intestinal immunity occurs before the migration of the larvae and the invasion of the muscle suggests that the host develops another phase of immunity during the muscle stage of the infection. This assumption is supported by the results of my previous work¹ in which I found that the serum from rabbits artificially immunized with dried, pulverized larvae has a marked parasiticidal effect when tested in vitro on the larvae, but little or no effect on the adults. The present experiments not only give further evidence of a specific antilarval and a separate antiadult humoral factor, but also give a reasonable explanation for the inability of many previous investigators to demonstrate an antibody basis for immunity to *Trichinella*.

LITERATURE REVIEW

The results of recent investigations have supplied evidence suggesting an

antibody basis for acquired immunity to *T. spiralis*. In studying the in vitro effect of immune serum on the trichina larvae and adults, the present author¹ demonstrated that precipitates form around the oral, anal and vulval openings of the adults and around the mouth of the larvae and are associated with the immobilization, disintegration and death of the worms. The precipitates are formed as a result of specific antibodies coming into contact with excretions and secretions of the worms. Mauss² described an in vitro precipitate around larvae, which is especially abundant when the larvae are incubated in immune whole serum and immune euglobulin and is less abundant when they are incubated in immune pseudoglobulin. Mauss³ also demonstrated that larvae incubated at 37 C. for 18 hours in 43% homologous serum are only 30% as infective as larvae which are incubated under the same conditions in normal serum. Roth⁴ confirmed the formation of precipitate around larvae incubated in human, guinea pig and rabbit immune serum, but he described no deleterious effects on the larvae and found that larvae incubated for 2 hours in immune serum are as infective as larvae incubated in normal serum or salt solution. McCoy⁵ reported that infective trichina larvae, fed to rats made resistant by previous infections, fail to develop in the intestine and are rapidly eliminated in the feces. On the other

Received for publication, April 16, 1941.

Mr. and Mrs. Frank G. Logan Fellow. This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

The author is indebted to Professor and Mrs. W. H. Taliaferro for advice in interpreting results and for aid in preparing the data for publication.

1. J. Infect. Dis. **67**: 292, 1940.

2. J. Parasitol., 1940, supp. 26, p. 43.

3. Am. J. Hyg., Sect. D, **32**: 80, 1940.

4. Acta path. et microbiol. Scandinav. **18**: 160, 1941.

5. Am. J. Hyg., Sect. D, **32**: 105, 1940.

hand, he failed to find any in vitro action of immune serum for periods up to 24 hours.

Roth⁶ found that an intestinal infection with a few hundred trichinae of either sex confers on guinea pigs a certain degree of protection against superinfection. Anderson and Leonard,⁷ using rats as experimental animals, confirmed Roth's findings. Rats, in which duodenal transplants of either male or female adults are made, develop a high degree of immunity although the infection is restricted to the intestinal stage.

Conflicting results have been reported on the passive transfer of acquired immunity to *T. spiralis*. Salzer⁸ reported that serum from patients recovering from trichinosis exerts a protective and curative action. Hall and Wigdor⁹ and Trawinski¹⁰ maintained that the protective property of immune serum is due to its antitoxic rather than to its parasiticidal action. Culbertson and Kaplan¹¹ found that mice are protected by injecting serum from rabbits obtained 8 weeks after a single infection. McCoy⁵ was unable to obtain passive transfer in rats using serum from superinfected animals.

MATERIALS AND METHODS

The rabbits consisted of males weighing from 2,500 to 3,000 gm. at the beginning of the experiments. The rats used were young adult males weighing from 90 to 125 gm. All animals were kept on standard diets.

Mature infective trichina larvae for the in vitro tests were obtained from rats which had been infected for at least

30 days. The larvae were separated from the muscles by artificial digestion in a pepsin hydrochloric acid mixture. The 5 day old larvae were those which escaped from the uteri of the females as a result of mechanical handling. The 10, 15 and 20 day old larvae were collected from rats infected an equivalent number of days by the maceration method described by Levin.¹² For convenience, the larvae from rats infected 30 days or longer will be referred to as mature or infective larvae, whereas those from rats infected 20 days or less will be called immature or migrating larvae. Adult worms for the in vitro tests and for test antigens were obtained from rats killed from the 5th to the 8th day after feeding infective larvae. The small intestine from the duodenum to the cecum was removed, opened and cut in small pieces about 1 cm. long. The sliced intestine was put in a Baermann apparatus with normal salt solution heated to 37 C. Most of the adults concentrated at the tip of the funnel during the first half hour and only a few remained attached to the intestine.

The test antigen for determining the precipitin content of all serums was prepared by making a 1% suspension of dried and well pulverized larvae or adults in Coca's alkaline solution (0.7% sodium chloride, 0.05% sodium bicarbonate and 0.4% phenol). Precipitin titrations were carried out by the "ring" test using undiluted serum overlaid with antigen dilutions beginning at 1:100 (1 gm. of powder extracted in 100 cc. of Coca's solution). Further dilutions were made as follows: 1:500, 1:1,000, 1:2,000, 1:3,000, 1:4,000 and 1:5,000. A few serums were positive in the dilution of 1:5,000. These were retested with dilutions up to 1:10,000. The tests were read after 1 hour at room temperature.

12. J. Parasitol., 1939, supp. 25, 24.

6. Am. J. Hyg., Sect. D, **30**: 35, 1939.

7. J. Parasitol., 1940, supp. 26: p. 42.

8. J. A. M. A. **67**: 579, 1916.

9. Arch. Int. Med. **22**: 601, 1918.

10. Zentralbl. f. Bakt. (Abt. 1), **134**: 145, 1935.

11. Parasitology **30**: 156, 1938.

The *in vitro* preparations were prepared as described in the previous paper.¹ The effect of the serums on the worms was measured by ascertaining the percentage of 50 worms which showed precipitates around their mouth, anus and vulva (this latter in adults only) and which showed immobilization, disintegration and death after incubation for the following periods of time: 1 and 12 hours, 1, 2, 3, 4, 5, 6 and 7 days. The amount of precipitate in the medium was also estimated.

Serums for the *in vitro* tests were obtained by bleeding rabbits aseptically from the marginal ear veins. When large amounts of serum were needed, animals were bled from the heart and their serum pooled. The bloods and serums were kept in the refrigerator at 6 C. without preservative. They were tested *in vitro* and *in vivo*, unheated, the day after being drawn. Serum from animals which received a single dose of larvae will be referred to as immune serum and that from superinfected animals will be called hyperimmune serum.

The number of infective larvae for the rat feedings and the number of adult worms recovered from the intestines were determined directly by counting. All feedings were made by stomach tube using slight ether anesthesia in the case of rats.

The standard error was calculated for the data in tables 3 and 5, but is not given in table 4 as there were only 5 rats in each group. The data in tables 3 and 5 are based on groups of only ten animals. Where the difference between two means was of the order of 3 times its standard error, the data were also calculated using for the standard deviation

$$\sigma' = \frac{\Sigma(d^2)}{(N-1)}$$

in which d represents the deviation of each observation from the mean and

N is the number of observations. The significance of the difference between two means as expressed by the difference divided by the standard error ($d/\sigma'd$) was then calculated by using Simpson and Roe's¹³ formula for t , which makes adjustments for the use of $N-1$.

EXPERIMENTAL RESULTS

1. The Antiparasitic Action of Serums *in Vitro*

Four rabbits (1, 2, 3 and 4) were fed an initial dose of 10,100 infective larvae. A fifth rabbit (control) was not infected. The 5 rabbits were bled on the fifth day previous to the day of feeding and on every fifth day throughout the course of infection. Beginning 70 days after the initial infection, 3 of the 4 rabbits (1, 2 and 3) were fed the following 5 additional doses of larvae: 10,400, 11,700, 12,200, 13,300 and 14,100, respectively, at 5 day intervals. During and after the superinfections of the 3 rabbits, all 5 of the rabbits were bled every fifth day for 50 days. The immune, hyperimmune and normal serums, thus obtained, were used for *in vitro* tests on adults and larvae and for precipitin tests with adult and larval test antigens.

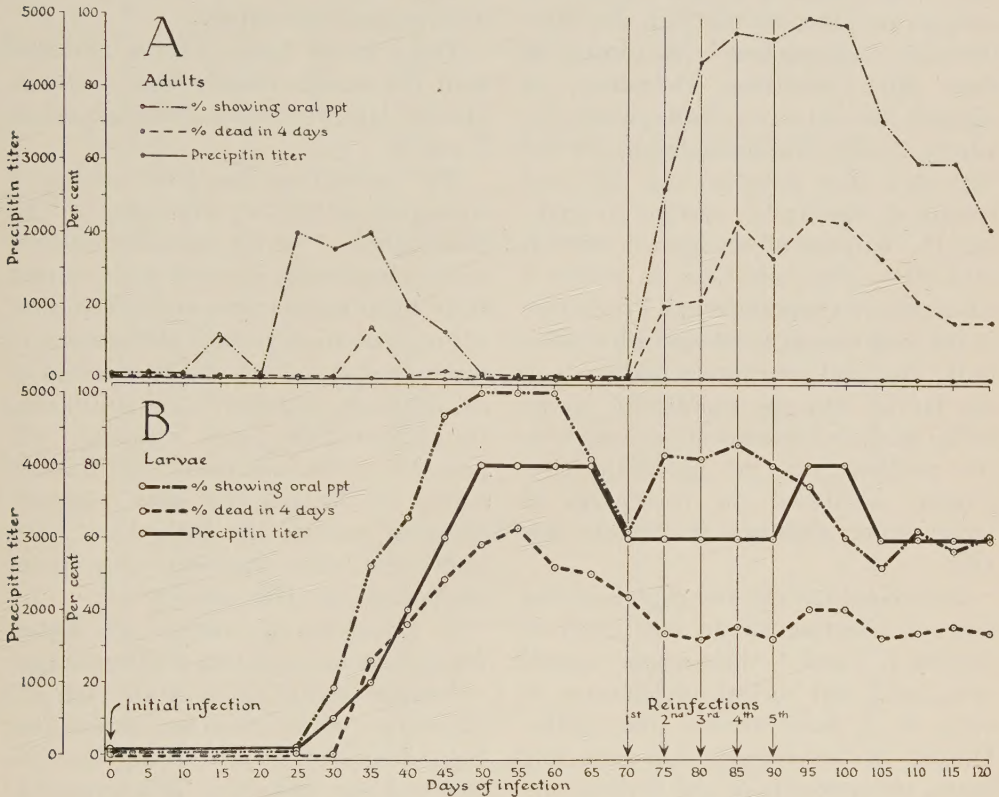
In several instances when the serums from the superinfected rabbits exhibited marked *in vitro* effects on both the adults and the larvae, the following additional tests were carried out. To one portion of serum, enough dried, pulverized larval material was added to make a 2% suspension. The mixture was stirred and incubated at 37 C. for 1 hour and then placed in the icebox at 6 C. overnight. The following morning the insoluble material was removed by centrifugation and the absorbed serum was used to determine its *in vitro* effect on

13. Simpson, G. G. and Roe, A.: Quantitative Zoology, New York and London, McGraw-Hill Book Co., Inc., 1939.

the trichina larvae and adults. The serum was tested for precipitins with adult and larval test antigens before and after absorption. Another similar portion of the serum was absorbed with pulverized adults and tested similarly.

a. *The antilarval and antiadult titer of serum from the rabbits during initial in-*

oral precipitate increased in amount in the in vitro preparations up to 24 hours. For 1 or 2 days thereafter the precipitate was often knocked off by the activity of the worms, but reappeared after longer incubation (cf. the percentage of worms with oral precipitate during the time from 1 hour to 7 days



Changes in the antilarval and antiadult antibodies in serum obtained from rabbit 1 during initial infection and subsequent superinfections. The antibodies were titrated by ascertaining with both adults and larva (1) the percentage of oral precipitate after incubation for 1 hour in vitro, (2) the percentage of worms dead in 4 days and (3) the precipitin titer by antigen dilution with dried, pulverized worm material.

fection and repeated superinfection.—Oral precipitate was seen after 1 hour when adults were incubated with serum taken as early as the 15th day after infection. After this period of time the potency of the serum increased until the 25th day, remained constant for about 10 days and then decreased to 0 by the 50th day of the infection (fig. 1A). The

in table 2). Anal and vulval precipitates appeared on the 30th day after infection, but they were never present on as many worms and did not appear in the in vitro preparations until the worms had been incubated 24 hours. Precipitate in the medium appeared on the 30th day after infection after 12 hours' incubation. It was not very heavy

during initial infection. The number of adults which died following the initial infection was relatively small (fig. 1A). The precipitin tests using dried pulverized adult worms as test antigen were consistently negative throughout this period (fig. 1A).

Oral precipitate was seen for the first time when larvae were incubated 1 hour with serum taken on the 30th day after infection and reached a maximum 50 days after infection. Thereafter, in marked contrast to the findings with the adults, it only decreased slightly by the 70th day after infection (fig. 1B) and remained practically constant throughout the balance of the period studied (120 days after infection) in rabbit 4 which was not superinfected. Precipitate in the medium occurred somewhat similarly. No anal precipitate occurred on the larvae, but the number of larvae which died, the amount of precipitate in the medium and the precipitin titer roughly paralleled the percentage of larvae found with oral precipitate (fig. 1B).

Five days after the first superinfecting dose of infective larvae was given to rabbits 1, 2 and 3, their serums caused oral, anal and vulval precipitates in vitro in 1 hour around the adults. During the subsequent superinfecting doses, the amounts of the various precipitates rose to higher levels than after initial infection (cf. the oral precipitate in fig. 1A after initial infection and after superinfection). Moreover, death of the worms occurred sooner and involved more worms than after initial infection (fig. 1A). No positive precipitin reactions were obtained with hyperimmune serums and dried pulverized adult worm test antigens (fig. 1A).

Superinfection did not significantly increase the action of the serums on the larvae as measured by the per cent of larvae showing oral precipitate, the

amount of precipitate in the medium and the number of larvae dead 4 days after incubation (fig. 1B). Furthermore, the precipitin content of the serum, as determined with the dried larval test antigen, did not rise to a higher level than that obtained during the initial infection (fig. 1B). No anal precipitate was found on the larvae when incubated in hyperimmune serum.

The data in figure 1 were obtained with the serums drawn from rabbit 1. Similar data were collected from rabbits 2 and 3.

The normal serums from rabbits 1 through 4 before they were infected and from rabbit 5 which was not infected were consistently inactive with respect to in vitro precipitates and precipitins.

For convenience, the 2 components of serum just described will be referred to as antiadult and antilarval antibodies throughout this paper although it is probable, on the one hand, that the two stages of the parasite have common antigens and, on the other hand, that each type may represent a group of antibodies (see Discussion).

b. The action of immune and hyperimmune serums on larvae of different ages.

—Immune serum taken 50 through 120 days after initial infection, as has just been described, has a strong in vitro effect on mature larvae. This serum has no such effect on the 5 day old larvae. It exercised a slight effect on the 10 day old larvae and a progressively increased effect on the 15 and 20 day old larvae. Thus, as may be seen in table 1, when serum from rabbit 4 was drawn 92 days after the initial infecting dose of larvae and tested in vitro, oral precipitate did not occur on the 5 day old larvae, occurred on a small number of the 10 day old larvae after 3 days of incubation and on a progressively greater percentage of the 15 and 20 day old larvae within a shorter incubation time. When this

TABLE 1.—*The in Vitro Action of Immune and Hyperimmune Serums* on Larvae of Different Ages as Ascertained by the Percentage of Larvae with Oral Precipitate, the Percentage of Dead Larvae and the Amount of Precipitate in the Medium.*
Note That Immune Serum Was Inactive on 5 Day Old Larvae and Became More Active as the Larvae Matured Whereas the Hyperimmune Serum Was Active on Larvae of All Ages

Immune Rabbit Serum: 1 Day Old, Unheated															
Precipitin titer with larval test antigen 1:3,000 and with adult test antigen O															
Age of Larvae	5 days			10 days			15 days			20 days			65 days		
	Larvae Showing Oral Precipitate %	Degree of Precipitate in Medium	Dead Larvae %	Larvae Showing Oral Precipitate %	Degree of Precipitate in Medium	Dead Larvae %	Larvae Showing Oral Precipitate %	Degree of Precipitate in Medium	Dead Larvae %	Larvae Showing Oral Precipitate %	Degree of Precipitate in Medium	Dead Larvae %	Larvae Showing Oral Precipitate %	Degree of Precipitate in Medium	Dead Larvae %
1 hr.	0	0	0	0	0	0	8	0	0	58	+	0	80	2+	0
12 hrs.	0	0	0	0	0	0	18	0	0	42	2+	0	74	4+	0
1 day	0	0	0	0	0	0	22	+	0	52	2+	0	68	4+	0
2 days	0	0	0	0	0	0	10	2+	0	54	2+	0	52	4+	0
3 days	0	0	0	6	0	0	12	2+	0	68	2+	0	54	4+	14
4 days	0	0	0	6	0	0	14	2+	0	60	2+	6	62	4+	20
5 days	0	0	0	6	0	0	8	2+	0	66	2+	10	68	4+	22
6 days	0	0	0	0	0	0	8	2+	0	62	2+	14	68	4+	24
7 days	0	0	0	0	0	0	4	2+	0	64	2+	18	70	4+	24

Hyperimmune Rabbit Serum: 1 Day Old, Unheated															
Precipitin titer with larval test antigen 1:4,000 and with adult test antigen O															
1 hr.	80	2+	0	62	+	0	88	+	0	78	2+	0	80	2+	0
12 hrs.	88	4+	0	70	2+	0	98	2+	0	80	2+	0	82	4+	0
1 day	76	4+	0	74	4+	0	94	4+	0	80	2+	0	80	4+	0
2 days	70	4+	0	60	4+	8	96	4+	6	72	4+	4	60	4+	0
3 days	72	4+	0	68	4+	0	96	4+	10	70	4+	24	66	4+	2
4 days	80	4+	10	74	4+	22	96	4+	24	66	4+	40	70	4+	6
5 days	86	4+	24	70	4+	38	96	4+	28	68	4+	42	72	4+	10
6 days	90	4+	36	66	4+	52	92	4+	36	62	4+	48	74	4+	16
7 days	94	4+	38	60	4+	46	94	4+	38	58	4+	58	76	4+	18

* No anal precipitate was found on any of the larvae when incubated in either immune or hyperimmune serum.

same serum was tested on 65 day old, mature larvae, oral precipitate occurred on 80% of the larvae after an incubation of 1 hour. No anal precipitate occurred on either the immature or mature larvae, but precipitate increased in the medium somewhat as did the oral precipitate on the various stages of the worm. Dead worms were only found among the 20 day old migrating stages. Similar results were obtained with immune serum taken from this same rabbit 103 days after infection.

The hyperimmune, unlike the immune, serum had marked in vitro effects on all of the migrating larvae as shown by the fact that as high as 80% of the 5 day old larvae showed oral precipitate 1 hour after incubation, 4+ precipitate occurred in the medium in 12 hours and 38% of the larvae were dead in 7 days (table 1).

c. *The specific absorption of hyperimmune serum with larval and adult antigens.*—Absorption tests were performed after superinfection, during which time the in vitro effect of the serum on the adults as well as on the larvae was marked. Table 2 presents evidence that the activity of hyperimmune serum was: 1) slightly absorbed with dried, pulverized larvae when tested in vitro on adults, 2) was entirely absorbed with similar larvae when tested in vitro on larvae, 3) was slightly absorbed with dried, pulverized adults when tested in vitro on adults and 4) was slightly absorbed with similar adults when tested in vitro on larvae. The slight amounts of absorption were probably due to the presence of small quantities of common worm antigens in the dried powder or to a nonspecific absorption by the powdered worm material. The absorption of the larval antibody with dried larvae, on the other hand, was complete. This finding makes it possible to obtain and test serum containing essentially the antiadult antibody alone.

2. The Antiparasitic Action of Serums in Vivo

For this experiment, 4 rabbits (6, 7, 8 and 9) were treated as was rabbit 1, and 3 rabbits (10, 11 and 12) were treated as was rabbit 4. These rabbits were then used to supply hyperimmune and immune serums for passive transfer experiments. In addition serum was obtained from artificially immunized rabbits 13, 14, 15 and 16 for a passive transfer experiment. Rabbits 13 through 16 were injected intravenously with 2 mg. of dried, pulverized larvae suspended in 10 cc. of sterile 0.85% salt solution on each of 3 days and, after 4 days, were given 3 similar injections. Their serums were then tested 3 days after the last injection for precipitin content as well as for their in vitro action on the trichina larvae and adults. Also normal serums from rabbits 5, 17, 18 and 19 were tested. The hyperimmune serum was drawn from the rabbits 6 hours after the fourth superinfecting dose. It was used fresh and after absorption with dried, pulverized larvae. The immune serum was drawn from rabbits 85 days after an initial infection at a time when it had a marked in vitro effect on the larvae and no effect on the adults. The serum from the artificially immunized rabbits was drawn 3 days after the last of 6 injections of dried, pulverized larvae, at a time when it had a marked in vitro effect on the larvae and no effect on the adults. These particular serums were used because, as was previously demonstrated (cf. fig. 1 and table 3), the untreated hyperimmune serum contained both the antiadult and antilarval antibodies, the hyperimmune serum absorbed with larvae essentially contained the antiadult antibody alone, the immune serum and serum from artificially immunized rabbits (cf. Oliver-González¹) essentially contained the antilarval antibody alone

TABLE 2.—*The in Vitro Action of Unabsorbed Hyperimmune Serum* and of Hyperimmune Serum* Absorbed with Larvae or with Adults on Adults and on Larvae. Note That the Hyperimmune Serum Absorbed with Larvae Did Not Form Precipitates around Larvae or Kill Them*

Time	Unabsorbed Hyperimmune Serum (Precipitin Titer with Larval Test Antigen, 1:3,000; with Adult Test Antigen, O)						Hyperimmune Serum Absorbed with Larvae (Precipitin Titer with Larval Test Antigen, O; with Adult Test Antigen, O)						Hyperimmune Serum Absorbed with Adults (Precipitin Titer with Larval Test Antigen, 1:3,000; with Adult Test Antigen, O)					
	Percentage Showing Precipitate			Degree of Precipitate in Medium		Dead Worms %	Percentage Showing Precipitate			Degree of Precipitate in Medium		Dead Worms %	Percentage Showing Precipitate			Degree of Precipitate in Medium		Dead Worms %
	Oral	Anal	Vulval				Oral	Anal	Vulval				Oral	Anal	Vulval			
Adults	94	0	14	2+	4+	0	74	0	8	+	+	0	96	0	24	2+	2+	0
	82	0	18	4+	4+	0	72	0	10	2+	2+	0	60	4	18	2+	2+	0
	84	4	22	4+	4+	0	74	0	14	2+	2+	0	48	6	16	2+	2+	0
	78	6	32	4+	4+	12	44	0	8	2+	2+	0	48	12	12	2+	2+	0
	76	14	34	4+	4+	18	54	2	10	2+	2+	0	50	18	14	2+	2+	6
	80	16	34	4+	4+	34	66	12	18	2+	2+	6	58	22	22	2+	2+	18
	84	18	36	4+	4+	38	74	14	18	2+	2+	10	62	28	28	2+	2+	22
Larvae	86	22	36	4+	4+	38	78	16	20	2+	2+	24	60	34	28	2+	2+	28
	90	24	36	4+	4+	38	72	18	24	2+	2+	24	62	36	32	2+	2+	30
	80	0		+	+	0	0	0		0	0	0	78	0		2+	2+	0
	80	0		4+	4+	0	0	0		0	0	0	72	0		4+	4+	0
	80	0		4+	4+	0	0	0		0	0	0	60	0		4+	4+	0
	78	0		4+	4+	8	0	0		0	0	0	42	0		4+	4+	0
	80	0		4+	4+	30	0	0		0	0	0	50	0		4+	4+	4
Larvae	80	0		4+	4+	32	0	0		0	0	0	68	0		4+	4+	16
	80	0		4+	4+	40	0	0		0	0	0	74	0		4+	4+	20
	74	0		4+	4+	56	0	0		0	0	0	70	0		4+	4+	26
	78	0		4+	4+	60	0	0		0	0	0	72	0		4+	4+	34

* All serums unheated, 1 day old.

and the normal serum contained neither.

a. *Passive transfer of acquired immunity to rats and the nonabsorbability of the protective property with larval antigen.*—Series 1 consisted of 50 rats divided into 5 groups of 10 rats each. Each of the 50 rats was fed with 1,000 mature trichina larvae. Ten of these rats received no other treatment, whereas 4 groups of ten rats each received 5 cc. of the following types of rabbit serum intraperitoneally 24 hours before and at the time of feeding: untreated hyperimmune serum, hyperimmune serum absorbed with powdered larvae, immune serum and normal serum. Five days after the day of feeding all of the rats were killed. The small and large intestines were removed, opened, sliced and placed in a Baermann apparatus for 1 hour, after which time the worms which had accumulated at the tip of the funnel were removed, washed in salt solution and counted. All the pieces of intestine were then removed from the apparatus and shaken vigorously in a volume of 50 cc. of 0.3% NaOH in 150 cc. flasks for 10 to 15 minutes. After centrifugation, the adult worms present in the sediment were counted and the figures added to the number of worms obtained in the Baermann apparatus.

Series 2 consisted of 25 rats divided into 5 groups of 5 rats each. These rats were each fed 1,000 trichina larvae and 4 out of the 5 groups were injected with the same types of serums in the same way as in series 1. The fifth group, as above, received no serum. Thirty-five to 39 days after feeding, the 25 rats were killed, and their carcasses without skin or entrails were digested in artificial gastric juice. The number of larvae freed from the muscle of each rat by this procedure was then recorded.

Series 3 consisted of 30 rats, each of which was fed 1,000 infective larvae. These were divided into 3 groups of 10

rats and, 24 hours before and at the time of feeding, each group received serum from the artificially immunized animals, normal serum and no serum, respectively. Five days after feeding, all of the rats were killed and the number of adult worms collected and counted as in series 1.

As may be seen from the data from the rats in series 1 (table 3), the numbers of adults recovered from the intestine of rats injected with hyperimmune serum were considerably less than the numbers of adults recovered from rats which received no serum or from rats which were injected with immune serum or normal serum. Thus, the difference between the worms recovered from the rats receiving the hyperimmune serum and the controls receiving normal serum was 336 ± 27 worms or 12 times the standard error. Even more significant differences were obtained in comparing the rats receiving hyperimmune serum with those receiving immune serum or no serum. Absorption of hyperimmune serum with dried pulverized larvae removed little, if any, of the protective antibody. Thus, the difference between the worms recovered from the rats which received normal serum and those which received absorbed hyperimmune serum was statistically still highly significant, i.e., 286 ± 30 worms or approximately 10 times the standard error. The protective property was not absorbed in spite of the fact that the precipitin titer was zero when tested with larval antigens. It may be, however, that absorption with the larval material removed some of the protective property although the difference between the worms recovered from rats which received the hyperimmune serum and those which received the absorbed serum was 50 ± 20 worms or 2.5 times the standard error. The statistical significance of this difference is very little

changed, i.e., $t=2.4$, when $(N-1)$ is substituted for N in computing the standard deviation. If further work demonstrates that there is a significant absorption, it might be due either to a nonspecific absorption or to the presence of common antigens in the larvae and adults, as previously pointed out in the case of the *in vitro* activity.

Although the groups of rats in series 2 were small, they corroborated the findings of series 1. The numbers of larvae obtained from the muscles of rats injected with hyperimmune serum and with hyperimmune serum absorbed with dried pulverized larvae were less than those obtained from rats injected with immune serum, normal serum or no serum. Thus, averages of 32,500 and 60,400 larvae were obtained from the rats given hyperimmune and absorbed hyperimmune serums, respectively, whereas averages of 173,600, 159,600 and 186,040 larvae were obtained from the rats given immune serum, normal serum and no serum, respectively (see component parts of table 4).

Statistically, no significant protection was found in rats receiving serum from rabbits artificially immunized with dried, pulverized larvae. Thus, an average of 376 ± 19 adult worms was obtained from the rats given serum from rabbits artificially immunized with powdered larvae, whereas averages of 430 ± 12 and 419 ± 22 adult worms were collected from rats given normal rabbit serum and no serum, respectively (see component parts of table 5). The differences between rats receiving serum from artificially immunized rabbits and the controls receiving normal rabbit serum (54 ± 22 worms) and no serum (43 ± 29 worms) are approximately 2.5 and 2 times their standard errors, respectively. When Simpson and Roe's value for t is used, i.e., when $(N-1)$ is substituted for N in the formula for the standard

TABLE 3.—*Passive Transfer of Acquired Immunity as Ascertained by the Number of Adults Recovered from the Intestines of the Rats.*
Note that Fewer Adult Worms were Recovered from the Intestine of Rats Given Hyperimmune Serum and to a Less Extent of Rats Given Hyperimmune Serum Absorbed with Larvae than from Rats Given Immune Serum, Normal Serum or no Serum

Unabsorbed Hyperimmune Serum (Precipitin Titer with Larval Test Antigen, 1:5000; with Adult Test Antigen, O)				Hyperimmune Serum Absorbed with Larval Powder (Precipitin Titer with Larval and Adult Test Antigens, O)				Immune Serum (Precipitin Titer with Larval Test Antigen, 1:4000; with Adult Test Antigen, O)				Normal Serum (Precipitin Titer with Larval and Adult Test Antigens, O)				No Serum			
Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total	Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total	Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total	Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total	Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total
1	86	9	95	11	116	20	136	21	352	80	432	31	316	96	412	41	418	41	459
2	73	2	75	12	88	36	124	22	407	81	488	32	411	74	485	42	501	20	521
3	41	11	52	13	170	17	187	23	501	107	608	33	312	101	413	43	356	42	398
4	140	0	140	14	190	7	197	24	290	96	386	34	226	99	325	44	391	70	461
5	70	16	86	15	56	40	96	25	204	212	416	35	202	142	344	45	390	10	400
6	49	21	70	16	271	19	290	26	304	113	384	36	309	94	403	46	407	7	414
7	52	4	56	17	41	39	80	27	304	106	410	37	220	108	328	47	416	26	442
8	29	10	39	18	91	5	96	28	276	87	366	38	466	110	576	48	470	13	483
9	139	10	149	19	100	70	170	29	302	80	382	39	344	154	498	49	497	4	501
10	66	10	76	20	160	31	191	30	286	81	367	40	309	80	389	50	329	92	421
Average			81 ± 12				131 ± 17				424 ± 23				417 ± 25				450 ± 13

TABLE 4.—*Passive Transfer of Acquired Immunity as Ascertained by the Number of Larvae Recovered from the Muscles of the Rats.**
Note that Fewer Larvae were Recovered from the Muscles of Rats Given Hyperimmune Serum and, to a Less Extent, of Rats Given Hyperimmune Serum Absorbed with Larvae than from Rats Given Immune Serum, Normal Serum or No Serum

Rat No.	Hyperimmune Serum (Precipitin Titer with Larval Test Antigen, 1:5000; with Adult Test Antigen, O)	Rat No.	Hyperimmune Serum Absorbed with Larvae (Precipitin Titters with Larval and Adult Test Antigens, O)	Rat No.	Immune Serum (Precipitin Titer with Larval Test Antigen, 1:4000; with Adult Test Antigen, O)	Rat No.	Normal Serum (Precipitin Titters with Larval and Adult Test Antigens, O)	Rat No.	No Serum
51	18,600	56	43,800	61	176,400	66	144,600	71	180,600
52	10,200	57	69,000	62	193,200	67	184,200	72	147,600
53	46,200	58	56,400	63	140,400	68	121,200	73	187,800
54	39,000	59	48,000	64	168,600	69	186,600	74	192,000
55	48,600	60	84,600	65	189,600	70	159,000	75	222,200
Average	32,500		60,400		173,600		159,600		186,040

* The rats were killed on the following days after infection: rats 51–55, 35 days; rats 56–60, 36 days; rats 61–65, 37 days; rats 66–70, 38 days; rats 71–75, 39 days.

deviation, the differences are 2.2 and 1.4 times their modified standard error. There is no question that the degree of protection is minor as compared to that produced by hyperimmune serum. If further work demonstrates that there is a partial protection with serum from rabbits artificially immunized with larval powder, however, this partial protection would be in accord with the partial absorption of the protective property from hyperimmune serum by dried, pulverized larvae.

The following conclusions seem war-

ranted by the data collected from the rats in series 1, 2 and 3: 1. Passive transfer is apparently mainly dependent on the antiadult antibody, since the action of hyperimmune serum absorbed with larvae, i.e., serum containing only the antiadult antibody, protected rats to such a large extent against infection as compared to the action of normal and no serum (cf. part 2 with parts 4 and 5 in tables 3 and 4). 2. Passive transfer is more or less independent of the anti-larval antibody since the action of immune serum, i.e., serum containing the

TABLE 5.—*Inconclusive Passive Transfer of Acquired Immunity as Ascertained by the Number of Adults Recovered from the Intestines of the Rats.*
Note that the Adults Recovered from the Intestine of Rats Given Serum from Artificially Immunized Rabbits are not Significantly Less in Number than from Rats Given Normal Rabbit Serum or no Serum

Serum from Artificially Immunized Rabbits (Precipitin Titer with Larval Test Antigen, 1:3000; with Adult Test Antigen, O)				Serum from Normal Rabbits (Precipitin Titters with Larval and Adult Test Antigens, O)				No Serum			
Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total	Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total	Rat No.	From Baermann Apparatus	By Treating Gut with NaOH	Total
76	313	12	325	86	502	3	505	96	242	41	283
77	406	21	427	87	408	17	425	97	440	52	492
78	274	19	293	88	320	44	364	98	413	60	473
79	420	14	434	89	299	54	353	99	473	11	484
80	324	76	400	90	410	40	450	100	490	3	493
81	320	61	381	91	470	12	482	101	370	13	383
82	417	41	458	92	460	9	469	102	346	20	366
83	411	9	420	93	399	11	410	103	356	14	370
84	284	10	294	94	418	10	428	104	370	17	387
85	311	20	331	95	416	2	418	105	414	49	463
Average			376 ± 19				430 ± 12				419 ± 22

antilarval antibody, was completely ineffectual in preventing the intestinal infection as compared to the action of normal and no serum (cf. part 3 with parts 4 and 5 in tables 3 and 4) and the absorption of hyperimmune serum with larvae removed at best only a small part of the protective property (cf. part 2 with part 1 in tables 3 and 4). 3. Since the antilarval antibody was so definitely inactive in immune serum, as just described, its perhaps minor involvement as indicated in certain of the data (absorption with larvae may have removed part of the protective activity in part 2 of tables 3 and 4 as compared to part 1 and serum from rabbits artificially immunized with larvae may have been slightly protective in part 1 of table 5 as compared to parts 2 and 3) may best be explained by assuming the presence of common antigens in the larvae and adults or by assuming a nonspecific absorption. This subject will be taken up in more detail in the discussion.

DISCUSSION

The results of the present investigation indicate that the serum of rabbits infected with *T. spiralis* contains two types of functional antibody which can be differentiated by (1) the stage of the parasite affected, (2) changes in titer throughout initial infection and superinfection, (3) relationship to the precipitin titer using adult and larval test antigens and (4) differences in absorption with the two stages of the parasite.

The titer of the antiadult antibody, as indicated by its in vitro effect on trichina adults, appeared 15 days after initial infection, was fairly high 25 to 35 days after infection, then dropped to zero by the 50th day, rose abruptly during superinfection and was highest at that time, whereas its precipitin titer using adult worms as test antigen was negative throughout initial infection

and superinfection. The titer of the antilarval antibody, on the other hand, as indicated both by its in vitro effect on mature trichina larvae and by its precipitin titer using larvae as test antigen, appeared 30 days after initial infection, was highest 45 to 60 days after infection and was not quite as high during superinfection. In addition, absorption with powdered adults of hyperimmune serum, i.e., serum in which both of the antibodies occurred in high concentration, did not markedly change either its in vitro activity or precipitin titer on adults¹⁴ or larvae, but its similar absorption with powdered larvae, removed its in vitro activity and precipitin titer on larvae and did not markedly change its in vitro activity and precipitin titer on adults.¹⁴ From these facts it is evident that serum containing essentially the antiadult antibody can be obtained by absorbing hyperimmune serum with larvae, whereas serum containing essentially the antilarval antibody can be obtained by collecting rabbit serum 50 days or more after initial infection at a time when the in vitro action of the serum is high against larvae and negative against adults. Serum containing essentially the antilarval antibody can also be obtained from rabbits artificially immunized with powdered mature larvae. By using such serums, it was ascertained that the antiadult antibody is evidently the important factor in passive transfer. The nonabsorbability of the in vitro effect with dried, powdered adults, however, makes it impossible to preclude the possibility that a third antibody, inseparable as yet from the antiadult

14. As will be recalled, the precipitin titer using adults as test antigen was consistently negative throughout and, hence, could not be affected by absorption, but precipitates did form around living adults in vitro. This latter activity was essentially unaffected by absorption.

antibody, may be responsible for the protective property of hyperimmune serum. If later work confirms the indication that serum from rabbits artificially immunized with powdered mature larvae (and hence containing only the antilarval antibody) actually partially protects rats from infection, and that this larval material actually specifically absorbs a portion of the protective property from hyperimmune serum, these findings will give an experimental basis for the presence of common antigens in adults and larvae. They would, however, be incompatible with the finding that immune serum taken 50 or more days after a single infection containing only the antilarval antibody is entirely lacking in protective titer. The latter finding may be due to questions of relative titer.

In contrasting the antiadult with the antilarval antibody, it is not intended to imply that there are only two qualitatively distinguishable antibodies arising as the result of two single dissimilar antigens. In the first place, each stage of the worm must contain many antigens, only a few of which are probably involved in the present study. In the second place, what have been termed larval and adult antigens in the present study are probably groups of antigens. As groups, these antigens react qualitatively differently, but there is no evidence that a given antigen is completely lacking in the other group. The qualitative difference between groups may be solely a question of the relative proportions of common antigens—a reasonable assumption which has some basis in the results discussed in the preceding paragraph. Whether due to changes in the relative proportions of common antigens or to the successive appearance of new antigens, the data demonstrate an interesting development of antigenic differences. Thus, as seen in table 1, the

antiadult, and not the antilarval, antibody is active against immature larvae at the time of their passage from the uterus. This fact appears to indicate that the immature larvae resemble the adult worms with respect to antigenicity and gradually acquire new antigenic characteristics as they mature. The basis for this change may be better understood when the nature of the worm antigens (probably excretions and secretions) is definitely known. The differences may be associated with the fact that the adults (and developing embryos) obtain their nourishment from the lamina propria of the gut, whereas the mature larvae obtain theirs from the muscle. In other words, enzymatic activities necessary in digesting different substrates may be correlated with the syntheses of certain antigens. These changes may be somewhat similar to the changes in the ability of certain lactobacilli to ferment sugars and the concomitant change in their polysaccharide antigens.¹⁵ Apropos of this discussion, Shtchoupakov¹⁶ has suggested that in addition to the defensive mechanism of the intestine, there is an immunity of the musculature. In the third place, the presence of common antigens in the adults and larvae best accounts for the suggestive, but statistically inconclusive, evidence that there is a slight amount of absorption by the adults of the *in vitro* effects on the larvae and by the larvae of the *in vitro* effects on the adults as seen in table 2, that there is a partial absorption of the antiadult antibody from hyperimmune serum by dried, powdered larvae as seen in part 2 of tables 3 and 4 and that there is a partial passive transfer of the antiadult effect in serum from rabbits artificially

15. Harrison, R. W.: *J. Infect. Dis.*, in press.

16. *Tr. Pasteur Inst. Epidemiol. & Bacteriol.* in Leningrad, Sect. Parasitol., 2: 231, 1935.

immunized with larvae as seen in part 1 of table 5.

The two types of antibody developed in trichinosis should be brought in line with previous studies on helminth infections, particularly with respect to (1) different types of functional antibodies arising during the course of infection as well as different types of antigens due to different stages of the parasite, (2) the nonabsorbability of certain protective antibodies, (3) the failure of some previous investigators to obtain *in vitro* effects and the passive transfer of acquired immunity and (4) various factors other than antibodies which have been postulated by previous workers to explain acquired immunity in trichinosis.

The finding of different antibodies which arise as different stages of the parasite develop in the present work most nearly approaches the results of Campbell.¹⁷ This investigator reported the formation of two types of antibodies in the serum of rats infected with the larval stage of *Taenia taeniaeformis*. The first type is associated with "early" immunity, is detectable within one week in infected animals and brings about the destruction of larvae before encystment. The second type of antibody is associated with "late" immunity, is detectable later in the infection and causes the destruction of the larvae after encystment. Furthermore, freshly ground worm material is an effective antigen for the first type of antibody in that such material absorbs this antibody from immune serum and incites the production of the antibody in normal animals. The same freshly ground worm material neither absorbs the second type of antibody from immune serum nor incites its production in normal animals.

The question of certain nonabsorb-

able protective antibodies has recently been reviewed by Taliaferro.¹⁸ This investigator pointed out that they may be only apparently nonabsorbable due to the fact that effective antigens are not present in sufficient quantities. Striking support of this viewpoint is given by the fact that the precipitin in serum, which produces precipitates around living adults and which probably acts parasitically *in vitro* and protectively *in vivo*, is so slightly absorbed by powdered adults. It is inconceivable that a precipitin is nonabsorbable as long as actual precipitates are formed. The fact that the living worm expels antigens which precipitate with this precipitin makes it likely, as suggested by Taliaferro, that the functional antigen may be completely destroyed during the death of the worms and the preparation of the test antigen or else, as suggested by Campbell,¹⁷ that it may only be liberated in sufficient quantities by the living parasite after a long sojourn in the host. The second explanation may hold true in this case because the larval test antigen prepared as was the adult test antigen did absorb the antilarval precipitin. The preparation of more concentrated suspensions of the dried, pulverized adults may, however, result in an efficient antigen. Experimental work on this particular point is in progress. There is a further possibility that the lack of positive precipitin tests with antigens prepared from adult worms and the similar lack of absorption of antiadult antibody with such material are due to the lack of optimal proportions in the tests. It should be noted that no matter what proves to be the basis for the absence of antiadult precipitin titer using dried adults as test antigens and the nonabsorbability of the antiadult antibody,

17. J. Immunol. **35**: 465, 1938.

18. Am. J. Trop. Med. **20**: 169, 1940; Physiol. Rev. **20**: 469, 1940.

other evidence in this investigation is sufficient for postulating an antiadult and an antilarval antibody (cf. especially fig. 1).

Several findings in the present work may help to explain the failure of various investigators to obtain passive transfer of acquired immunity to or the *in vitro* action of immune serum against *T. spiralis*. In most previous work, the serums used for passive transfer were obtained from animals which had only received an initial infection and at a period too long after infection, i.e., at a time when the antiadult antibody content of the serum is low. In other instances, the serum from rats and guinea pigs, which do not produce as potent antibodies as rabbits, were used. Thus, Schwartz¹⁹ reported negative passive transfer experiments with serum from a rabbit four months after infection. Also McCoy's⁵ negative results may have been due to the fact that he used rat serums. The observations of Roth⁴ on the *in vitro* action of immune serum were discontinued after 24 hours, a period of time which according to my results is too short to observe some of the deleterious effects. The failure of McCoy⁵ to find precipitates around the larvae may have been due to the fact that the worms in whipping about may knock off the precipitate, especially if they are not confined in a small space.

Various factors have been postulated in acquired immunity in trichinosis. McCoy⁵ maintains that it is unlikely that humoral antibodies play an important part in the mechanism of immunity to trichinae. He believes that the mechanism of resistance is chiefly mechanical in that the sensitized mucosa in immune animals secretes an increased amount of mucus, which with increased peristalsis effects a rapid ex-

pulsion of the worms. Chandler²⁰ has postulated two types of immunity in various intestinal nematode infections which he correlates with the position of the worms in the host. According to him the first or local intestinal immunity, which he considers the most important in trichinosis, is stimulated by antigens which do not reach the blood stream in appreciable quantities, but are absorbed by the local mucosal cells so that various generalized manifestations of immunity, such as serum antibodies, do not appear. The second or general immunity, which is of minor importance in trichinosis, arises when the parasites live parenterally and, hence, distribute antigen into the blood stream with the consequent occurrence of circulating antibodies and other generalized manifestations of immunity. Taliaferro,²¹ on the other hand, believes that the so-called parenteral and local immunities are due fundamentally to the same mechanisms, one of which is dependent on antibodies. He¹⁸ also emphasizes that the lack of parallelism between the titer of a selected antibody and immunity to parasitic invasion does not necessarily indicate a lack of the functional role of antibodies in immunity unless it is first demonstrated that the antibody selected for study and the immunity are incited by the same antigen.

The present investigation clarifies some of these points. As shown by Bachman and Rodríguez-Molina²² and as emphasized by Chandler,²⁰ repeated superinfection results in immunity to superinfection, which is largely expressed by the inability of new adults to parasitize the intestine, and yet, circulating precipitins do not increase as tested with larval antigens. The present work verifies this basic finding and indi-

20. *Am. J. Trop. Med.* **19**: 309, 1939.

21. *J. Parasitol.* **20**: 149, 1934.

22. *Am. J. Hyg.* **18**: 266, 1933.

19. *J. A. M. A.* **69**: 884, 1917.

cates that the seeming lack of parallelism is due to the fact that these investigators were dealing with the antiadult antibody in immunity and were testing the antilarval antibody in vitro. Actually strengthening the hypothesis that antibodies do take part in the intestinal phase of immunity is the fact that antiadult antibodies actually increase with repeated superinfection. Taliaferro and Sarles²³ and Taliaferro¹⁸ have pointed out another difficulty in assuming that a local intestinal immunity exists which is not associated with antibodies and is fundamentally different from parenteral immunity. In both *Nippostrongylus* and *Trichinella*, the worms in the intestine pierce the epithelium and, on the one hand, pour excretions and secretions into the lamina propria and, on the other hand, cause the removal of fluid which may include antibodies. In a physiological sense, therefore, since the worm is in close contact with the blood stream, these infections actually are parenteral. To Taliaferro, the differences between the local and general manifestations of acquired immunity are not fundamental. In the case of antibodies, the differences arise from the interplay, on the one hand, of such factors as the local formation and spread of antibodies and the associated degree of localization and spread of antigens, and, on the other hand, of such factors as the recruitment of antibodies from the blood stream into local areas of inflammation.

SUMMARY

Two types of antibody appear in the serum of rabbits as a result of infection with *T. spiralis* which act specifically on the adults and on the larvae, respectively. The antiadult type causes precipitates at the mouth, anus and vulva

and sometimes the death of the adults in vitro. The oral precipitate is most common and appears more quickly in vitro. The antilarval antibody causes oral, but no anal, precipitate and sometimes the death of the larvae in vitro. Precipitate also occurs free in the medium. In addition, the antiadult antibody appears to be the active factor in passive transfer.

Each of the two antibodies is probably a composite of several or more antibodies arising as a result of the presence of several or more antigens in each stage of the worm. There is suggestive, but inconclusive evidence, that common antigens occur in larvae and adults. The apparent qualitative differences between the two stages may be due to quantitative differences, i.e., the proportional amount of common antigens may vary in the two stages. Within the admittedly narrow scope of the present tests, the larvae in the uterus are antigenically similar to the adults and become differentiated to the antigen composition of the so-called mature larvae after 20 days.

The antiadult type of antibody, as indicated by its in vitro effect on adults, appears about the 15th day after infection, is moderately high between 25 and 35 days after infection and then decreases to zero by the 50th day, it reappears around 5 days after superinfection and reaches its highest titer during repeated superinfections, it partially protects rats against the intestinal stage of infection (passive transfer), it is only slightly, if at all, absorbed with dried, powdered adults, and its precipitin titer as tested with such powdered material is zero during initial infection and superinfection. These last two attributes may not be an inherent characteristic of the antibody. They may rather be due to the absence of functional antigen in the tests.

23. J. Infect. Dis. 64: 157, 1939.

The antilarval type of antibody, as indicated by its *in vitro* effect on larvae, appears around the 30th day after infection, is highest from 45 to 60 days after infection and only decreases slightly during the subsequent 60 days, its titer is not increased but remains moderately high during repeated super-infections, it gives little or no protection

to rats against the intestinal stage of the infection (lack of passive transfer), it is absorbed with dried, powdered larvae, it is produced in rabbits artificially immunized with dried, powdered larvae and the precipitin titer using such larval material somewhat parallels its *in vitro* effect on the larvae.

